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# Metabolism and metabolic inhibition of cilnidipine in human liver microsomes<sup>1</sup>

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**KEY WORDS** cilnidipine; metabolism; cytochrome P-450; liver microsomes

## ABSTRACT

**AIM:** To study the metabolism of cilnidipine and the effects of selective cytochrome P-450 (CYP450) inhibitors on the metabolism of cilnidipine in human liver microsomes *in vitro*. **METHODS:** Human liver microsomes were used to perform metabolism studies. Various selective CYP450 inhibitors were used to investigate their effects on the metabolism of cilnidipine and the principal CYP450 isoform involved in dehydrogenation of dihydropyridine ring of cilnidipine in human liver microsomes. **RESULTS:** Cilnidipine was rapidly metabolized to three metabolites. They are dehydrogenated metabolite of dihydropyridine ring of cilnidipine (M<sub>1</sub>), demethylation metabolite of lateral chain of dihydropyridine ring of cilnidipine (M<sub>2</sub>), and the dehydrogenation and demethylation metabolite of cilnidipine (M<sub>3</sub>). Ketoconazole (Ket) competitively inhibited the dehydrogenation of dihydropyridine ring of cilnidipine and lowered the metabolic rate of cilnidipine while  $\alpha$ -naphthoflavone ( $\alpha$ -Naph), sulfaphenazole (Sul), quinidine (Qui), diethyldithiocarbamate (DDC), and tranylcypramine (Tra) had a little or no inhibitory effects on the dehydrogenation of cilnidipine. **CONCLUSION:** Cilnidipine was rapidly metabolized in human liver microsomes and dehydrogenation of dihydropyridine ring of cilnidipine is crucial for the elimination of cilnidipine. Cytochrome P-4503A (CYP3A) is the major human CYP involved in the dehydrogenation of dihydropyridine ring of cilnidipine.

## INTRODUCTION

Cilnidipine is a novel dihydropyridine calcium antagonist and its calcium antagonistic activity is lasting longer than those of nifedipine and nicardipine<sup>[1]</sup>. It has been reported that cilnidipine has been used for the treatment of hypertension and hypertensive-associated vas-

cular disorders<sup>[2]</sup>.

After oral administration, large amount of the drug could be detected in the gallbladder, bladder, liver and kidney. Approximately 18 %-29 % and 80 % of the dose was excreted in urine and feces, respectively within 72 h in dogs<sup>[3]</sup>. The purpose of this experiment was to investigate the metabolism of cilnidipine in human liver microsomes *in vitro* and the effects of selective CYP450 inhibitors on the metabolism of cilnidipine in human liver microsomes and the major CYP450 isoform involved in the metabolism of cilnidipine.

## MATERIALS AND METHODS

**Chemicals** Cilnidipine (CNDP) and its dehydro-

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generated metabolite (DCNDP) were kindly provided by Department of Pharmaceutical Chemistry, China Pharmaceutical University. Glucose-6-phosphate dehydrogenase (Type V),  $\alpha$ -naphthoflavone ( $\alpha$ -Naph), sulfaphenazole (Sul), tranlycypromine (Tra), quinidine (Qui), diethyldithiocarbamate (DDC) were purchased from Sigma Chemical Co. Ketoconazole (Ket) was kindly provided by Nanjing Second Pharmaceutical Factory.  $\alpha$ -Nicotinamide adeninedinucleotide phosphate (NADP) and glucose-6-phosphate (G-6-P) were purchased from Shanghai Lihudongfeng Biotechnological Co. All other supplies were of the highest grades available from standard commercial sources.

**Tissue samples and preparation of liver microsomes** The human liver used in the present study was obtained from Jinling Hospital after the study protocol was approved by the Academic Committee. Microsomes were prepared by differential centrifugation<sup>[4]</sup>. Microsomal protein concentration was determined by the method of Lowry *et al*<sup>[5]</sup>.

**Incubation and sample preparation** The incubation conditions of the experiment were established and controlled to provide a reproducible and linear rate of the metabolite. A typical incubation mixture<sup>[6]</sup> consisted of potassium phosphate buffer (pH 7.4) 100 mmol/L, an NADPH generating system (MgCl<sub>2</sub> 5 mmol/L, G6P 10 mmol/L, NADP 1 mmol/L, G6PDH 1 kU/L), cilnidipine 25  $\mu$ mol/L, and microsomal protein 1 g/L, in a final volume of 1 mL. The reaction was initiated by addition of the NADPH generating system. After incubation at 37 °C for 40 min, the reaction was terminated by adding 200  $\mu$ L of NaOH 2 mmol/L. CNDP and DCNDP formed in the incubation mixture were determined by HPLC method described previously<sup>[7]</sup> with minor modification. The reaction mixtures were extracted with 4 mL of ether-hexane (1:1) and centrifuged at 1000 $\times$ g for 5 min. The organic fraction was evaporated under a gentle stream of air at 50 °C. The residue was dissolved in 100  $\mu$ L of mobile phase and an aliquot (20  $\mu$ L) was injected onto the Hypersil BDS column (4.6 $\times$ 250 mm, 5  $\mu$ m). The HPLC system comprised of an LC-10AT pump and a SPD-10AV UV detector.

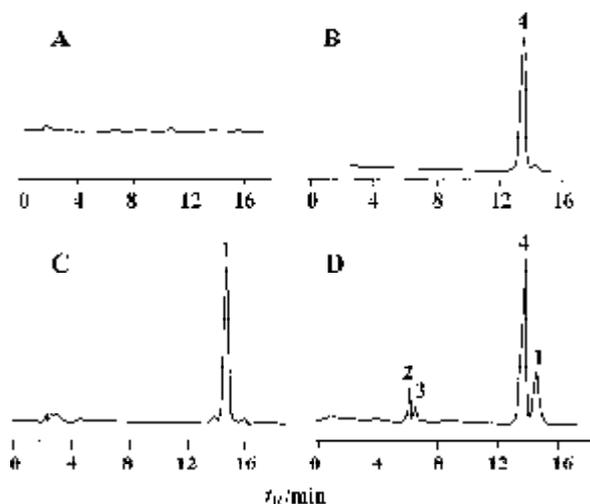
**Inhibition study** The effects of various selective CYP inhibitors<sup>[8]</sup> on the dehydrogenation of dihydropyridine ring of CNDP in human liver microsomes were investigated. The inhibitors studied were  $\alpha$ -Naph (CYP1A2), Qui (CYP2D6), DDC (CYP2E), Sul (CYP2C9), Tra (CYP2C19) and Ket (CYP3A). The concentration of CNDP was 25  $\mu$ mol/L and the con-

centration range of inhibitors was 2.5-20  $\mu$ mol/L for Qui, 12.5-100  $\mu$ mol/L for  $\alpha$ -Naph, Sul and Tra, 6.25-50  $\mu$ mol/L for DDC, and 0.5-5  $\mu$ mol/L for Ket.

## RESULTS

Following incubation of CNDP with human liver microsomes CNDP was rapidly metabolized and three metabolites (M<sub>1</sub>, M<sub>2</sub>, and M<sub>3</sub>) were isolated (Fig 1) in the incubation with the corresponding concentration of CNDP decreased. Direct HPLC/MS analysis using an electrospray ionization interface under positive ion mode resolved three drug-related compounds in the incubations (Fig 2). M<sub>1</sub>, M<sub>2</sub>, and M<sub>3</sub> exhibited the protonated molecular ion [M+H]<sup>+</sup> at *m/z* 491, 479 and 477 respectively while CNDP exhibited the protonated molecular ion [M+H]<sup>+</sup> at *m/z* 493. Both HPLC and mass spectra with prepared reference substance conformed that M<sub>1</sub> was dehydrogenated metabolite of dihydropyridines ring of CNDP. The other two metabolites, M<sub>2</sub> and M<sub>3</sub>, were tentatively identified as the demethylation metabolite of lateral chain of dihydropyridine ring of CNDP (M<sub>2</sub>) and the dehydrogenation and demethylation metabolite of CNDP (M<sub>3</sub>), respectively according to their LC/MS data. The proposed metabolic pathway of CNDP is present in Fig 3.

The effects of inhibitors on the formation of DCNDP are presented in Fig 4. Ket, the specific inhibi-



**Fig 1.** HPLC chromatograms of (A) blank human liver microsomes, (B) cilnidipine standard, (C) dehydrogenated metabolite standard, and (D) incubation of cilnidipine with human liver microsomes. Peak 1: dehydrogenated metabolite; peak 2: demethylated metabolite; peak 3: dehydrogenated and demethylated metabolite; and peak 4: cilnidipine.

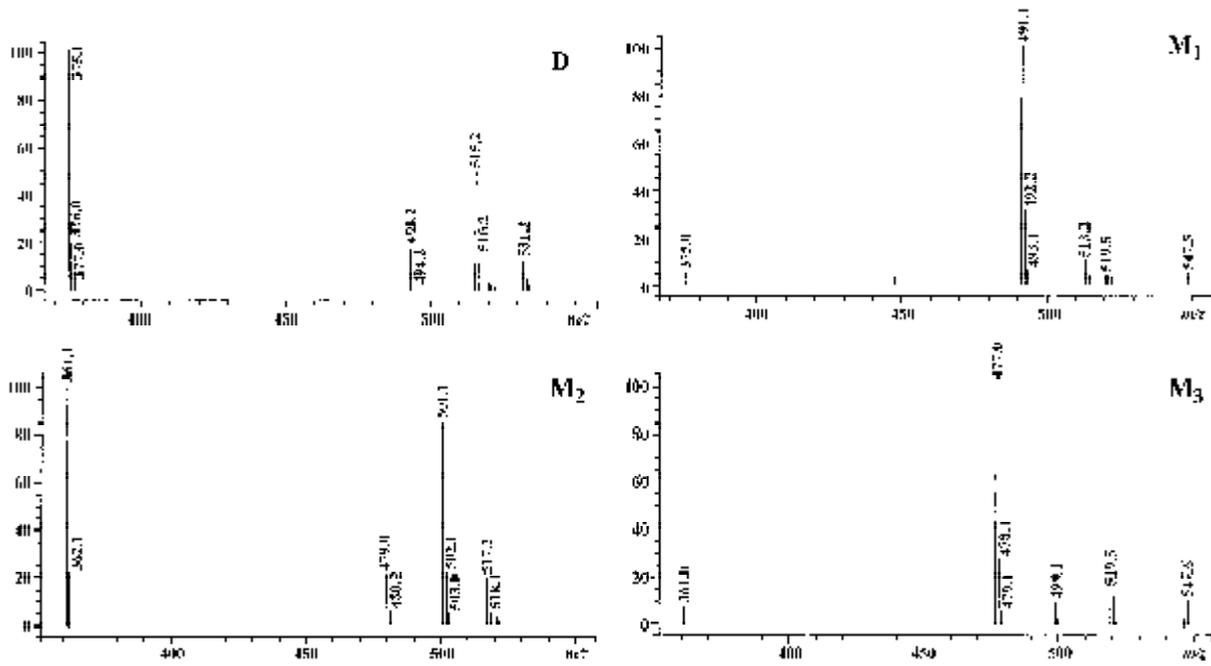


Fig 2. LC/MS spectra of cilnidipine and its metabolites in human liver microsomes.

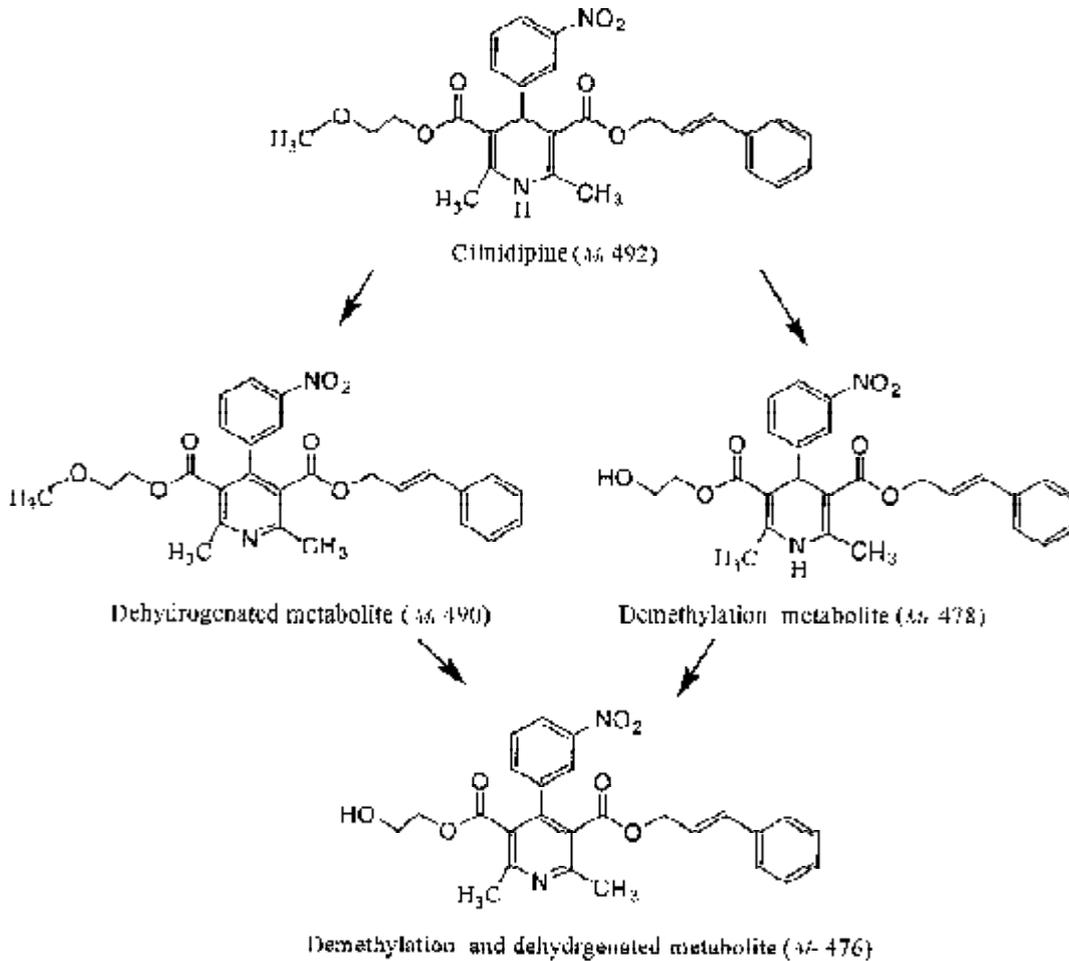


Fig 3. The proposed metabolic pathway of cilnidipine in human liver microsomes.

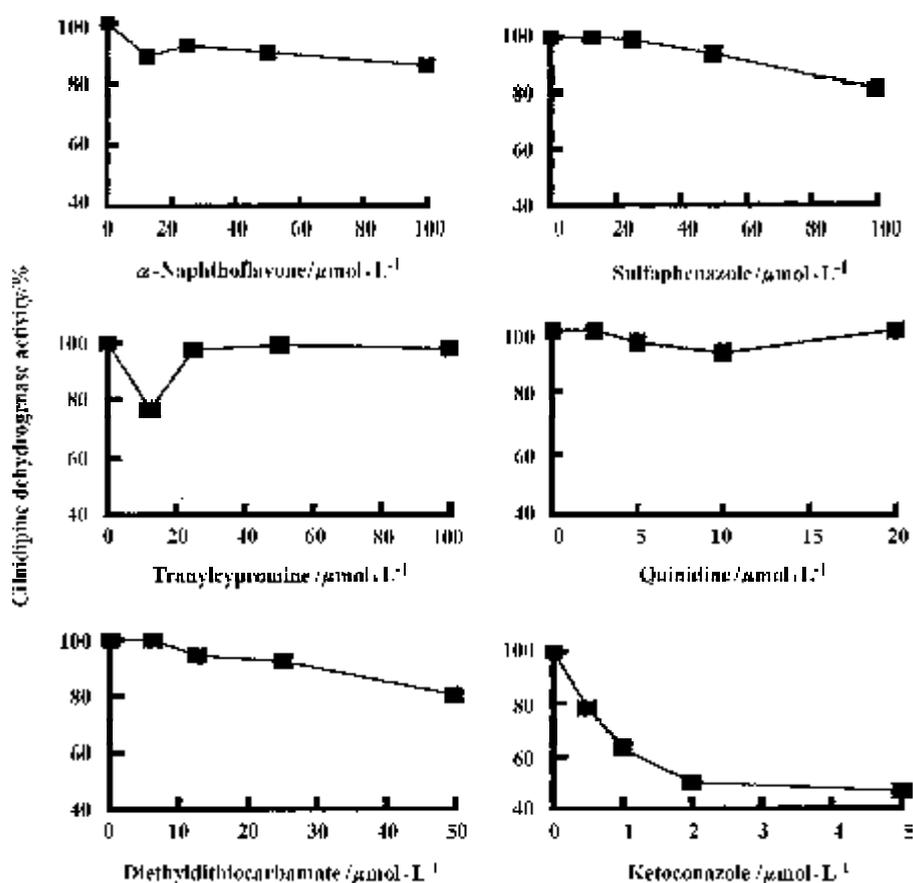


Fig 4. Effects of CYP450 selective inhibitors on cilnidipine (25 mmol/L) dehydrogenase activity in human liver microsomes. Effect of each inhibitor was compared with the control values determined by the incubation of cilnidipine alone and expressed as the percentage of control values (100 %).

tor of CYP3A, could inhibit the formation of DCNDP while other inhibitors had no significant inhibitory effect on the DCNDP formation as shown in Fig 4. The Lineweaver-Burk plot of cilnidipine dehydrogenase activity in human liver microsomes in the absence or presence of ketoconazole is presented in Fig 5. Ket competitively inhibited the formation of DCNDP and lower the rate of CNDP metabolism in human liver microsomes (Fig 6).  $K_i$  value for Ket was 1.52  $\mu\text{mol}$ .

## DISCUSSION

The results of present studies indicated that cilnidipine was rapidly metabolized in human liver microsomes. Three metabolites were isolated and identified in the incubation mixture. Dehydrogenated metabolite of dihydropyridine ring of CNDP was one of its major metabolites in the incubation. Inhibition of the formation of this metabolite will directly result in lowering of the metabolic rate of CNDP. This suggested

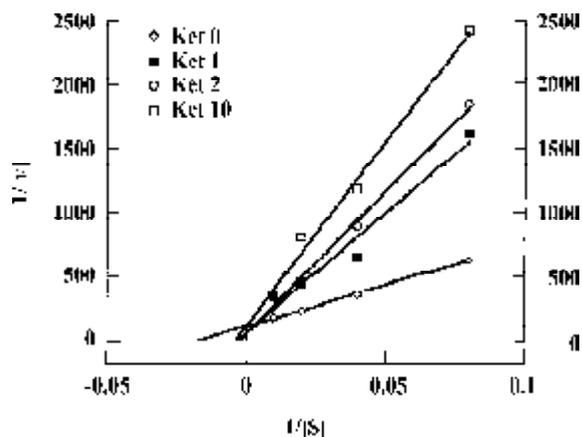
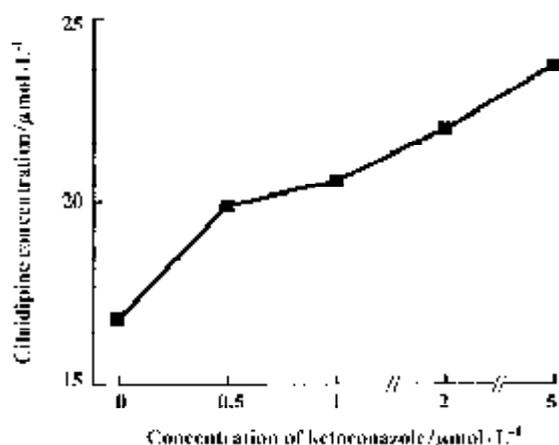


Fig 5. Effect of ketoconazole on cilnidipine dehydrogenase activity in human liver microsomes. Each data point represents the mean of duplicate determination.  $[v]$ : reaction velocity ( $\text{mmol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ ),  $[S]$ : substrate concentration ( $\text{mmol}\cdot\text{L}^{-1}$ ).

that dehydrogenation of dihydropyridine ring of CNDP was the initial metabolic step and this step is crucial for



**Fig 6.** Effect of ketoconazole on the metabolism of cilnidipine (25 mmol/L) in human liver microsomes. Each point represents the mean of duplicate determination.

the elimination of CNDP. Our previous studies<sup>[9,10]</sup> have shown that the initial and crucial metabolic step of both nimodipine and felodipine, which are also dihydropyridine calcium channel blockers, was dehydrogenation of dihydropyridine ring in human liver microsomes. The results of our previous studies and present study suggested that dehydrogenation of dihydropyridine ring of dihydropyridine calcium channel blockers was the crucial metabolic step for the elimination of this type of drugs and the therapeutic agents which can inhibit the above metabolic step of calcium channel blockers will lower the metabolic rate of this type of agents and elevate the level of this type of agents in the body. This is worth noticing in clinic when they are used in combination with other therapeutic agents which may have the metabolic interactions with them.

Ket, the specific inhibitor of CYP3A, could competitively inhibit the formation of DCNDP and lower the metabolism of cilnidipine, while other inhibitors for CYP1A2 ( $\alpha$ -Naph), CYP2C9 (Su1), CYP2C19 (Tra), CYP2D6 (Qui), and CYP2E (DDC) had no or a little effect on the formation of DCNDP. The results implicated that CYP3A as a major CYP450 isoform involved in the dehydrogenation of dihydropyridine ring of cilnidipine. Pharmacokinetic drug interactions caused by metabolic processes are regarded as one of the most important factors that affect the concentration of drugs

in the body<sup>[11]</sup>. Therefore, inhibition of the above metabolic pathways can lower the rate of cilnidipine metabolism and increase the concentration of cilnidipine in the body, suggesting the possibility of the metabolic interactions of cilnidipine with other therapeutic agents, inhibitors of CYP3A. These results contribute to our understanding the metabolism of CNDP in human.

## REFERENCES

- 1 Yoshimoto R, Dohmoto H, Yamada K, Goto A. Prolonged inhibition of vascular contraction and calcium influx by the novel 1, 4-dihydropyridine calcium antagonist cilnidipine (FRC-8653). *Jpn J Pharmacol* 1991; 56: 225-9.
- 2 Watanabe K, Hosono M, Dozen M, Hiruma T, Hayashi Y. Effect of FRC-8653, a  $\text{Ca}^{2+}$  channel blocker, on stroke and hypertensive complications in stroke-prone spontaneously hypertensive rats (SHRSP) [abstract]. *Jpn J Pharmacol* 1993; 61 (Suppl 1): 118.
- 3 Mealy N, Castaner J. Cilnidipine. *Drugs Future* 1996; 21: 249-53.
- 4 Vonbahr C, Groth CG, Jansson H, Lundgren G, Lind M, Glaumann H. Drug metabolism in human liver *in vitro*: establishment of a human liver bank. *Clin Pharmacol Ther* 1980; 27: 711-25.
- 5 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. *J Biol Chem* 1951; 193: 256-75.
- 6 Kimonen T, Juvonen RO, Alhava E, Pasanen M. The inhibition of CYP enzymes in mouse and human liver by pilocarpine. *Br J Pharmacol* 1995; 114: 832-6.
- 7 Liu XQ, Ren YL, Qian ZY, Wang GJ. Enzyme kinetics and inhibition of nimodipine metabolism in human liver microsomes. *Acta Pharmacol Sin* 2000; 21: 690-4.
- 8 Newton DJ, Wang RW, Lu AYH. Cytochrome P450 inhibitors. Evaluation of specificities in the *in vitro* metabolism of therapeutic agents by human liver microsomes. *Drug Metab Dispos* 1995; 23: 154-8.
- 9 Liu XQ, Ren YL, Peng JH, Qian ZY, Wang GJ. Metabolism of nimodipine in human liver microsomes. *Chin Pharmacol Toxicol* 2000; 14: 449-52.
- 10 Zhao Y, Liu XQ, Qian ZY, Fan XL, Ren YL. Enzyme kinetics and inhibition of felodipine metabolism in human liver microsomes. *J China Pharm Univ* 2001; 32: 112-6.
- 11 Nakajima M, Inoue T, Shimada N, Tokudome S, Yamamoto T, Kuroiwa Y. Cytochrome P450 2C9 catalyzes indo methacin *O*-demethylation in human liver microsomes. *Drug Metab Dispos* 1998; 26: 261-6.